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EXAMINER	
AUDET, MAURY A	
ART UNIT	PAPER NUMBER
1654	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/596,077	MANTELATTO ET AL.	
	Examiner	Art Unit	
	MAURY AUDET	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *3/8/10*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-40,43 and 44* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-40,43 and 44* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *27 May 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *3/8/10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment and response are acknowledged. **All rejections have been overcome, but for the outstanding 103 rejection, now made Final (necessitated by amendment as to claim 44).**

As discussed below, the primary amendment based on the Examiner's review of the record (alongside arguments) is the modification of the minimum molecular weight from 750,000 Da (which the art was cited as teaching previously) to 850,000.

The Examiner is open to interviewing the application (103 rejection, skilled artisan routine optimization grounds) should Applicant find this of interest/value, after receipt of this Office Action.

The present application has been transferred from Examiner Underdahl to the present Examiner.

The Invention

As noted in the previous action, claims 1-40 and 43-44 are now pending an examined on the merits, after amendment.

Claim 1 and 44 are the only two independent claims.

Claim 44 is a single product claim, a product by process, of producing the known compounds polyhydroxyalkanoates (PHAs, such poly-3-hydroxybutyrate (PHB) or poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV)) by the method of claim 1.

Claim 1 contains the remaining 40 dependent claims drawn to various optimization of materials, amounts, ranges, etc. for use in the 6 method steps of making PHA's.

As generally discussed before, claims 1-40 are drawn to a method of making:

1. (Original) A process for recovering polyhydroxyalkanoates (PHAs) from cellular biomass of bacteria, said biomass being obtained by fermentation and in the form of a cellular biomass slurry in aqueous suspension and with a dry cellular content not inferior to about 18% by weight, characterized in that it comprises the steps of:

i) submitting the concentrated cellular biomass slurry to concomitant operations of injection of PHA solvent, of vigorous agitation and of quick heating in the interior of a reactor, in order to provoke the rupture of the walls of the cellular biomass and the dissolution of the PHA contained in the latter, and to form a suspension comprising PHA solvent enriched with dissolved PHA, water remaining from the cellular biomass slurry and insoluble residues of the concentrated cellular biomass;

ii) submitting the suspension formed in the reactor to a separation step, for recovering the solvent, enriched with the dissolved PHA, from the insoluble residues of the remaining cellular biomass;

iii) rapidly cooling the PHA solvent solution enriched with PHA to a temperature which is sufficient to substantially precipitate all the dissolved PHA;
iv) cold micro-filtrating the PHA suspension precipitated in the PHA solvent containing water and impurities dissolved therein, in order to separate a concentrated paste of precipitated PHA;

v) submitting the paste concentrated with PHA to simultaneous operations of washing with water, heating and agitation, in order to promote the evaporation of a certain amount of solvent which is adequate to obtain a suspension containing PHA granules of high porosity and which are brittle and easily shearable, the remaining solvent, and water;

vi) submitting the washed and heated PHA granules to agitation and shearing, so as to rapidly break them, while processing the extraction of the residual solvent by injecting water vapor into the suspension containing the remaining solvent and water, in order to obtain purified PHA particles in the suspension; and

vii) separating the purified PHA particles from the suspension.

The Disposition of the International Authority

The International Authority found the same (or nearly identical) claims 1-44 as having both novelty and inventive step. Reciting that:

2.1 CITATIONS

Reference is made to the following documents:

D1:
NONATO R VET AL: "Integrated production of biodegradable plastic, sugar and ethanol", APPL. MICROBIOLOGY & BIOTECHNOLOGY, vol. 57, no. 1-2, October 2001, pages 1-5

D2:
ROSSELL C E V ET AL: "Production of biodegradable plastic (PHB), sugar and ethanol in a sugar mill" INTERNATIONAL SUGAR JOURNAL, vol. 104, no. 1243, 2002, pages 321-323

2.2 NOVELTY (Art. 33(2) PCT)

D1 and D2 disclose a process for recovering PHB from cellular biomass of bacteria obtained by fermentation, comprising steps of thermal inactivation, dilution with water, flocculation, separation and concentration, and multi-step extraction with medium-chain-length alcohols (D1 page 2 right-hand column line 35-59, Table 1, and page3 right-hand column line 10-37; D2 page 321 center column). *The process(es) disclosed in D1 and D2 yield PHB characterized by the parameters in Table 1 in both documents.* The *process as disclosed and claimed in independent claim 1 of the current application differs from the process(es) disclosed in D1 and D2 by its sequence of steps, in particular steps* ***involving (vigorous) agitation and (quick) heating (step i), (rapid) cooling (step iii), (cold) microfiltration (step iv), and washing, heating and agitation (step v).*** *The resulting product has a molecular weight higher than the one disclosed in Table 1 of D1 and D2, as well as advantageous properties in terms of smell and color.*

Hence, the present application satisfies the criterion set forth in Article 33(2) PCT as the subject-matter of claims 1-44 appears to be new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.3 INVENTIVE STEP (Art. 33(3) PCT)

The present application also satisfies the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1-44 is considered to involve an inventive step (Rule 65(1)(2) PCT). In view of D1 and D2, which are considered as closest prior art to the subject-matter of independent claims 1 and 44, *the problem to be solved can be summarized as the provision of methods* ***for recovering PHA yielding products that are of high purity and of high molecular weight,*** *environment-friendly and cost-effective.* The solution as claimed in claim 1 is not obvious in view of the prior art. ***Although individual steps of the recovering process may lack novelty and/or inventive step, the combination of steps is considered to involve an inventive step****, and yielding a product with advantageous properties that could not be anticipated to result from the process of claim 1.*

As noted previously, notwithstanding the disposition of the International Authority, or casting any doubt thereon based on their view of their art of record – the present action relying on different art of record – each and every application is examined on its own merits. The IA did not provide the benefit of identifying which step(s), with quantifiable limitation thereto, took the individually known methods steps out of lacking inventive step (obviousness), to become, in combination, novel.

Firstly, the steps cited above by the IA (under 33(2)) as not taught in the same sequence/combination are nevertheless known steps in PHA/PHB/PHVB recovery, e.g.: ***(vigorous) agitation and (quick) heating (step i), (rapid) cooling (step iii), (cold) microfiltration (step iv), and washing, heating and agitation (step v).*** **Furthermore, the terms modifiers of these steps (vigorous, quick, rapid, cold) thereto are subjective, non-quantifiable limitations.**

Second, this Examiner none of the claims are found to claim a purity amount (as cited by the IA under 33(3)), and only the final dependent claims are drawn to weight/size ranges of the particles that may be recovered.

Third, this Examiner is not yet convinced that such weight/size ranges, absent something more (e.g. unexpected results thereof, or in the way purity), could not have been arrived at depending on the desired size preferred by the skilled artisan using the same or similar methods of making.

Claim Rejections - 35 USC § 102-Vacated Following Amendment to Claim 44; Claim 44 Now Rejected Under 103, Necessitated by Amendment

The rejection previously stated:

Claim 44 is a product by process. Where the product is found in the art, prior to a presently claimed product thereto, how said product is made does not bear patentable weight. A product remains a product, however made.

NOTE: It cannot be ascertained whether the 5 art references recited above expressly teach each and every process step (6) and dependent variations thereof, of the claimed product by process of claims 1-43. Thus, the latter rejection is applied under a 35 USC 102/103 over the same references of record.

Claim 44 is rejected under 35 U.S.C. 102(b) as being anticipated by the following 5 references that recovered the same polyhydroxyalkanoate (PHA) species products of specifically poly-3-hydroxybutyrate (PHB) or poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV) (Applicant's claims 5 and 7), by applying solvents and other steps/materials to recover the same from a bacteria biomass:

1. **Horowitz et al. I (US 6323276)-BOTH PHB & PHBV**
2. **Horowitz et al. II (US 6228934 B1)-BOTH PHB & PHBV**
3. **Horowitz et al. III (US 6368836 B2) -PHB**
4. **Traussnig et al. (US 4,968,611) (cited in Horowitz et al. III, '836 B2)-PHB**
5. **Blauhut et al. (US 5,213,976) (cited in Horowitz et al. III, '836 B2)-PHB**

1. **Horowitz et al. I (entire document)** teach :

col. 4:

"The PHAs can be derived from biological systems including bacteria and genetically engineered plant crops. In bacterial systems, the PHAs are accumulated intracellularly as granular inclusion bodies. PHA also can be produced in genetically engineered plant crops. Methods for recovering PHAs from plant biomass are described, for example in PCT WO 97/15681, PCT WO 97/07239, and PCT WO 97/07229. The methods described herein similarly are useful with a variety of PHAs, regardless of source organism or comonomer composition. Representative PHAs include poly-3-hydroxybutyrate (PHB), poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV, marketed as BIOPOL.TM. by Monsanto), poly-3-hydroxybutyrate-co-4-hydroxybutyratepoly-3-hydroxypropionate, poly-3-hydroxybutyrate-co-3-hydroxypropionate, poly-4-hydroxybutyrate, poly-3-hydroxybutyrate-co-3-hydroxyhexanoate, poly-3-hydroxybutyrate-co-3-hydroxyoctanoate, poly-5-hydroxyvalerate, and poly-6-hydroxyhexanoate."

2. **Horowitz et al. (US 6228934 B1) (entire document)** teach:

e.g. col. 4:

The PHAs can be derived from biological systems including bacteria and genetically engineered plant crops. In bacterial systems, the PHAs are accumulated intracellularly as granular inclusion bodies. PHA also can be produced in genetically engineered plant crops. Methods for recovering PHAs from plant biomass are described, for example in PCT WO 97/15681, PCT WO 97/07239, and PCT WO 97/07229. The methods described herein similarly are useful with a variety of PHAs, regardless of source organism or comonomer composition. Representative PHAs include poly-3-hydroxybutyrate (PHB), poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV, marketed as BIOPOL.TM. by Monsanto), poly-3-hydroxybutyrate-coA-hydroxybutyratepoly-3-hydroxypropionate, poly-3-hydroxybutyrate-co-3-hydroxypropionate, poly-4-hydroxybutyrate, poly-3-hydroxybutyrate-co-3-hydroxyhexanoate, poly-3-hydroxybutyrate-co-3-hydroxyoctanoate, poly-5-hydroxyvalerate, and poly-6-hydroxyhexanoate.

3. **Horowitz et al. (entire document)** teach:

"Method of decolorizing or deodorizing polyhydroxyalkanoates from biomass with ozone" (title);

"**Methods for the recovery and cation of polyhydroxyalkanoates (PHAs)from biomass containing PHAs**, wherein the methods include treating the biomass or partially

purified PHA with ozone, in at least one step of a purification process, have been developed. **Treatment of PHA-containing biomass or partially purified PHA with ozone yields an enhanced level of purity suitable for coating and other applications**. The ozone treatment also has the added advantage that be resulting PHA polymer or polymer latex is essentially odor-free. The ozone treatment may be used alone or in combination with other treatment, extraction, and separation stages, and is especially suitable for the treatment of PHA-containing latexes slurries, suspensions, and organic solutions" (abstract)

col. 1:

Polyhydroxyalkanoates (PHAs) are thermoplastic polyesters which can be produced from bacteria or plants (Williams & Peoples, CHEMTECH 26:33-44 (1996)). These polymers can be recovered from the biological systems (the biomass) by organic solvent processes, aqueous processes, or a combination of both organic solvent/aqueous processing. Examples of known organic solvent recovery processes are described in U.S. Pat. No. 4,310,684 and No. 4,705,604 to Vanlautem et al. (extraction of PHB from microbes with chlorinated solvents); **U.S. Pat. No. 4,968,611 to Traussnig et al. (use of diols, acetalized triols, di- or tricarboxylic acid esters or butyrolactone to extract poly-3-hydroxybutyrate (PHB) and its copolymers from microbes)**; **U.S. Pat. No. 5,213,976 to Blauhut et al. (process for extracting PHB from microbial cells using methylene chloride followed by precipitation of the PHB in water)**; PCT WO 97/15681; PCT WO 93/11656 (use of acetone to extract poly-3-hydroxyoctanoate polymer from Pseudomonas oleovorans); PCT WO 96/06179 and PCT WO 97/15681 (solvent methods for recovering PHAs from transgenic plant crops); and U.S. Pat. No. 5,821,299 to Noda (the use of solvent/partial non-solvent mixtures for extracting PHAs from biomass). Typically, in each of these prior art processes, some of the biomass components are co-extracted with the PHA, which can cause the PHA product to be discolored and/or to have an unpleasant odor.

4. Traussnig et al. (entire document) teach:

As cited in Horowitz et al. III, Traussnig et al. teach the "**use of diols, acetalized triols, di- or tricarboxylic acid esters or butyrolactone to extract poly-3-hydroxybutyrate (PHB) and its copolymers from microbes**"

The key sections of p. 1-4 of Traussnig et al. teach:

Extracting agents for poly-D(-)-3-hydroxybutyric acid (title)

Use of diols or acetalized triols, di- or tricarboxylic acid esters,

mixtures of dicarboxylic acid esters or butyrolactone as extracting agents for obtaining pure polyesters or copolyesters containing 3-hydroxybutyric acid units. (abstract)

[ ]

Poly-D(-)-3-hydroxybutyric acid (poly-HB) is synthesized and accumulated inside the cell by many microorganisms as a substance for storing energy and carbon and represents a polyester having thermoplastic properties which is biologically degradable. Poly-HB can, for example, be prepared in good yields without problems by the procedures described in U.S. Pat. No. 4,786.598.

Copolyesters of poly-HB, such as, for example, copolyesters consisting of 3-hydroxy-butyric acid and 3-hydroxyvaleric acid units and also other acid units should, according to EP-A-0,052,459, exhibit better processing properties than pure poly-HB when used as thermoplastics. A process for the preparation of such copolyesters is disclosed in EP-A-0,069,497.

[ ]

In U.S. Pat. No. 3,275,610, chloroform is described as an extracting agent. In order to achieve good yields, however, the cells must be treated for a very long time with the extracting agent. However, due to the long treatment, depolymerization of the poly-HB occurs so that either a poor yield or a reduction in the molecular weight of poly-HB must be taken into account in this method.

In U.S. Pat. No. 4,310,684, other halogenated hydrocarbons are proposed for the extraction. However, halogenated hydrocarbons are on the whole toxic and represent a hazard for any who have to work with them, and additionally a pollution of the environment, it also having to be taken into consideration that residual contents of this solvent in the isolated poly-HB are unavoidable.

In U.S. Pat. No. 4,101,533, cyclic carbonic acid esters such as ethylene carbonate or propylene carbonate were therefore proposed as solvents for polyhydroxybutyric acid. However, these solvents are very corrosive in the hot state in which they have to be used and attack taps and joints of apparatuses. A relatively long treatment of the cells with ethylene carbonate or propylene carbonate is necessary for a good yield in the extraction of poly-HB, a particularly large reduction in the molecular weight of the poly-HB or its copolyesters occurring, however, which has disadvantageous consequences for the use of poly-HB or its copolyesters as thermoplastics.

In contrast, solvents for the simple and problemfree extraction of poly-HB

and its copolyesters could now be found, the use of which as extracting agents avoids the abovementioned disadvantages, the polyesters or copolyesters being obtained in an unexpected high purity of at least 98% in very good yields.

[ ]

100 g of a water-moist cell material from the fermenter, obtained by removing the fermenter solution by centrifugation and having a water content of 60% by weight and a poly-HB content of 78% relative to the cell dry weight, were stirred for 10 minutes at 140.degree. C. with 360 g of 1,2-propanediol. After separating the undissolved cell material by means of a heated suction filter, the solution was cooled, whereupon poly-HB gelled. The precipitated gel was filtered off with suction, stirred well with water and then washed, whereupon the gel crystallized, and the crystalline precipitate was filtered off with suction and dried. In this way, 24.6 g of poly-HB, which corresponds to 79% of theory, having a purity of 99.1% and a molecular weight of 585,000 were obtained, the molecular weight of the poly-HB in the cell material being 650,000.

[ ]

25 g of a water-moist cell material from a fermenter, obtained by removing the fermenter solution by centrifugation, and having a water content of 60% by weight and a poly-HB content of 65% relative to the cell dry weight, were stirred at 120.degree. C. for 15 minutes with 390 g of glycerol formal. After separating the undissolved cell material by means of a heated suction filter, the solution was cooled, whereupon poly-HB gelled. The precipitated gel was filtered off with suction and then washed with water and acetone, whereupon the gel crystallized, and the crystalline precipitate was filtered off with suction and dried. **In this way, 5.5 g of poly-HB, which corresponds to 85% of theory, having a purity of 99.7% and a molecular weight of 700,000 were obtained, the molecular weight of the poly-HB in the cell material being 780,000.**

[ ]

25 g of a water-moist cell material from the fermenter, obtained by removing the fermenter solution by centrifugation, and having a water content of 60% by weight and a poly-HB content of 62% relative to the cell dry weight, were stirred for 15 minutes at 110.degree. C. with 390 g of diethyl succinate. After separating the undissolved cell material by means of a heated suction filter, the solution was cooled, whereupon poly-HB gelled. The precipitated gel was filtered off with suction, then washed with water and ethanol,

whereupon the gel crystallized, and the crystalline precipitate was filtered off with suction and dried. In this way, 5.6 g of poly-HB, which corresponds to 90% of theory, having a purity of 100% and a molecular weight of 400,000 were obtained, the molecular weight of the poly-HB in the cell material being 470,000.

[ ]

25 g of a water-moist cell material from the fermenter, obtained by removing the fermenter solution by centrifugation, and having a water content of 60% by weight and a poly-HB content of 62% relative to the cell dry weight, were stirred for 15 minutes at 110.degree. C. with 390 g of dimethyl succinate. After separating the undissolved cell material by means of a heated suction filter, the solution was cooled and methanol was added, whereupon poly-HB was precipitated. The deposited precipitate was filtered off with suction, then washed with water and acetone and dried. In this way, 5.3 g of poly-HB, which corresponds to 86% of theory, having a purity of 99.9% and a molecular weight of 420,000 were obtained, the molecular weight of the poly-HB in the cell material being 470,000.

[ ]

25 g of a water-moist cell material from the fermenter, obtained by removing the fermenter solution by centrifugation, and having a water content of 60% and a poly-HB content of 62% relative to the cell dry weight, were stirred for 15 minutes at 120.degree. C. with 390 g of a mixture consisting of dimethyl succinate:dimethyl glutarate:dimethyl adipate in the ratio 1:4:1. After separating the undissolved cell material by means of a heated suction filter, the solution was cooled and ethanol was added, whereupon poly-HB precipitated. The precipitate was filtered off with suction, then washed with ethanol and dried. In this way, 5.5 g of poly-HB, which corresponds to 89% of theory, having a purity of 98.9% and a molecular weight of 725,000 were obtained, the molecular weight of the poly-HB in the cell material being 780,000.

[ ]

125 g of a water-moist cell material from the fermenter, obtained by removing the fermenter solution by centrifugation, and having a water content of 60% by weight and a poly-HB content of 62% relative to the cell dry weight, were stirred at 110.degree. C. for 15 minutes with 450 g of butyrolactone. After separating the undissolved cell material by means of a heated suction filter, the solution was cooled, whereupon the poly-HB gelled. The precipitated gel was filtered off with suction and then washed with water and acetone, whereupon the gel crystallized, and the crystalline precipitate was

filtered off with suction and dried. In this way, 28 g of poly-HB, which corresponds to 90% of theory, having a purity of 99.5% and a molecular weight of 735,000 were obtained, the molecular weight of the poly-HB in the cell material being 780,000.

[ ]

1. In a process for the preparation of a polyester or copolyester containing 3-hydroxybutyric acid units by cultivation of a poly-D(-)-3-hydroxybutyric acid producing microorganism in a fermentation medium and subsequent extraction and recovery of the desired product, the improvement which comprises using as an extracting agent a solvent selected from the group consisting of diols and acetalized triols, di- or tricarboxylic acid esters, mixtures of dicarboxylic acid esters, and butyrolactone to obtain said polyester or copolyester in substantially pure form.

5. Blauhut et al. (US 5,213,976)

As cited in Horowitz et al. III, **Blauhut et al. teach a process for extracting PHB from microbial cells using methylene chloride followed by precipitation of the PHB in water.**

Process for extracting polyhydroxyalkanoates from the cell material of microorganisms by adding an organic solvent for the polyhydroxyalkanoate which is immiscible with water and which has a boiling point of below 100.degree. C., and, if appropriate, by adding water; stirring the resulting extraction mixture, if appropriate with refluxing; separating off the aqueous phase which contains the cell material in undissolved form from the organic phase; and injecting the organic phase into hot water, causing the dissolved polyhydroxyalkanoate to precipitate and the organic solvent to evaporate, and also isolating the precipitated polyhydroxyalkanoate flocs. (abstract)

[ ]

Polyhydroxyalkanoates, in particular homopolymers and copolymers of D-(-)-3-hydroxybutyric acid (poly-HB) are synthesized and accumulated intracellularly by many microorganisms as a storage substance for energy and carbon, and they represent polyesters which have thermoplastic properties and which are biodegradable. Poly-HB can be prepared in good yields with the aid of microorganisms, for example following the procedure described in U.S. Pat. No. 4,786,598 or U.S. Pat. No. 4,957,861. Copolyesters of poly-HB such as, for example, copolyesters which consist of 3-hydroxybutyric acid units and 3-hydroxyvaleric acid units or, alternatively, other acid units, can be prepared by way of fermentation, for example by one of the procedures described

in EP-A-0 052 459, EP-A-0 204 442, EP-A0 288 908, EP-A-0 304 293 or EP-A-0 274 151. The polyhydroxyalkanoates formed are integrated into the cell material of the microorganism and must be separated from the cell material, which is relatively difficult. One possible way of separation is extraction with the aid of a solvent, but carrying out the processes described to date also presents difficulties.

[ ]

In a preferred embodiment, the poly-HB-containing cell material is separated from the fermentation solution by centrifugation, whereupon such an amount of methylene chloride and, if appropriate, such an amount of water is added to the cell material that the ratio by weight of cell material relative to the cell dry weight:water:methylene chloride is approximately 1:3:10 to 1:5:30. The resulting mixture is either subsequently refluxed for 10 to 60 minutes, with stirring, or stirred at room temperature with the aid of a dynamic high-speed stirrer, and, if appropriate, cooled and centrifuged, in which process an aqueous phase containing the cell residues in undissolved form and an organic phase containing the poly-HB in dissolved form are formed. The methylene chloride phase is withdrawn from the aqueous phase and, with the aid of steam, injected into a heated container into which water has been introduced at a temperature of from 70.degree. to 90.degree. C., with stirring. The poly-HB which is thereby caused to flocculate in the water is stirred in the hot water for 20 to 40 minutes, separated off with the aid of a centrifuge and dried at 80.degree. to 100.degree. C. in a tray oven.

[ ]

60 liters of an aqueous fermentation solution containing 26% by weight of a cell material of Alcalicenes latus with a poly-HB content of 72% by weight, obtained by the procedure described in EP-A-0,144,017 and after removing part of the fermentation solution by centrifugation by means of a disc separator with solids discharge, were treated with 30 1 of water and 400 1 of methylene chloride and refluxed for 30 minutes with stirring. The resulting mixture was centrifuged at 2200 rpm in a syphon centrifuge with a drum diameter of 630 mm, during which process an aqueous phase containing the cell material of the microorganism in undissolved form and an organic phase containing poly-HB in dissolved form, were formed. The organic bottom phase was withdrawn from the aqueous top phase and injected into 800 l of water at a temperature of 80.degree. C. which had been introduced into a stirred container, by means of a two-component nozzle with a 4 mm diameter bore for the PHB solution and an annular gap width of approx. 2 mm for the propellant, namely steam, at an admission pressure of 3 bar, using a volume stream of 300 1/h. During this process, the temperature of the water was maintained approximately constant

with the aid of a heating jacket which surrounded the container. During this process, poly-HB precipitated in the form of flocs, while the methylene chloride and a small amount of the water evaporated and was condensed and collected outside the container. When injecting had ended, the suspension was stirred for 30 minutes at 80.degree. C. The content of the container was subsequently pumped into a trailing-blade centrifuge with a drum diameter of 630 mm, and separated into water and centrifuge-moist poly-HB flocs at a centrifuge speed of 2000 rpm. The centrifuge-moist flocs were dried for 24 hours in a tray drier at 80.degree. C.

[ ]

This gave 9.5 kg of poly-HB, which is 85% of theory, of a purity of >99% and a methylene chloride content of <1 ppm.

[ ]

50 ml of an aqueous fermentation solution as described in Example 1 were treated with 20 ml of water and 350 ml of methylene chloride, and the mixture was stirred for 2 minutes at room temperature with the aid of an Ultra Turrax high-speed stirrer manufactured by IKA, Maschinenbau, Janke & Kunke GmbH, Germany. In this process, 97% by weight of the poly-HB which had been present in the cell material dissolved.

[ ]

2. The process according to claim 1, wherein the polyhydroxyalkanoate is a homopolymer or copolymer of poly-D(-)-3-hydroxybutyric acid.

Claim Rejections - 35 USC § 103-Maintined

(102 side dropped after Applicant's Amendment)

The rejection of claims 1-40 - and now 44 following amendment of primary note the modification of the minimum molecular weight from 750,000 Da (former claim 42; which the art of Traussnig et al. taught) to 850,000 Da - is maintained for the reasons of record.

Applicant's arguments and amendments have been considered but are not found persuasive.

As discussed above, and relying merely by example on 4. Traussnig et al. (entire document), Traussnig et al. teach a level of purity of 98% or > of a PHA/PHB with minimum molecular weight of 750,000 Daltons. Although Applicant has deleted this limitation (former claim 42) and raised the bar to 850,000 minimum, as the 103 rejection previously closed with and once again is maintained, nothing would have precluded the routine optimization of by the skilled artisan to reach the same purity (e.g. 98-99.9%) at slightly higher Da amount (850,000). Purity level at a high Da amount, in the view of the Examiner, remains the focus of the end product of claim 44 - by way of the method of claim 1. The other modifications to the claims are simply deemed routine optimization in light of the what is known in the art.

The previous 103 rejection is repeated below for continuity of record:

Though drawn to a process of producing a "purified PHA" provides no positively claimed limitations directed to an end product purity (e.g. %). Thus, absent evidence to the contrary, nothing would have precluded the products in the art from being purified to the same degree, that apriori, is not claimed in the present invention.

It is also noted that the claims are drawn to the "comprising" transition phrase, which would allow any other steps herewith (e.g. using ozone as part of purification; as in Horowitz et al.: "Methods for the recovery and cation of polyhydroxyalkanoates (PHAs)from biomass containing PHAs, wherein the methods include treating the biomass

or partially purified PHA with ozone, in at least one step of a purification process, have been developed" (abstract)).

Claims 1-43 are rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over the following 5 references that the substantive steps of recovering a polyhydroxyalkanoate (PHA) product of specifically poly-3-hydroxybutyrate (PHB) or poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV) (Applicant's claims 5 and 7) by applying solvents and other materials to recover the same from a bacteria biomass:

1. Horowitz et al. I (US 6323276)-BOTH PHB & PHBV
2. Horowitz et al. II (US 6228934 B1)-BOTH PHB & PHBV
3. Horowitz et al. III (US 6368836 B2) -PHB
4. **Traussnig et al. (US 4,968,611) (cited in Horowitz et al. II, '836 B2)-PHB**
5. Blauhut et al. (US 5,213,976) (cited in Horowitz et al. III, '836 B2)-PHB

The 5 references are fully discussed above. *AS NOTED ABOVE: It cannot be ascertained whether the 5 art references recited above expressly teach each and every process step (6) and dependent variations thereof, of the claimed product by process of claims 1-43. Thus, the latter rejection is applied under a 35 USC 102/103 over the same references of record.*

It would have been obvious to one of ordinary skill in the art at the time the invention was made to follow the same steps, or make routine optimizations thereof (solution, solvents, amounts/ranges of materials used, etc.) to recovere the polyhydroxyalkanoate (PHA) species products of specifically poly-3-hydroxybutyrate (PHB) and/or poly (hydroxybutyrate-co-

hydroxyvalerate) (PHBV) in any of Horowitz et al. I-III, Traussnig et al., or Blauhut et al., because each of the references advantageously teach the same or routinely optimizable steps of recovering the same polyhydroxyalkanoate (PHA) species products of specifically poly-3-hydroxybutyrate (PHB) and/or poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV).

Absent evidence to the contrary of some unexpected increased production, speed of recovery, or purity of the same end products, any steps using routinely optimizable solutions, solvents, amounts/ranges of materials used, etc. known in the art would have predictably produced the same products, in the same or similar amounts/purity and thus been obvious.

Likewise, as to claims 41-43 specifically, nothing would have precluded the skilled artisan from recovering/isolating the PHA/PHB/PHBV at any obtainable weight (e.g. 850,000 Da) or particle average size (e.g. 40-400pm; 100-200pm) thereof, absent evidence to the contrary of some unexpected result obtained by using this size v. any other size; e.g. a product using the same v. the same product using another size.

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the reference, especially in the absence of evidence to the contrary.

Prior Art Made of Record But Not Relied Upon

As cited before:

The following 5 references are cited of record, but not relied upon, which though not expressly teaching the isolation of the PHA species of poly-3-hydroxybutyrate (PHB) or poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV), do expressly teach the method of making/recovering PHA's generally. [The 3 references applied taught the preferred species recovery/product, thus the other 3 references were not deemed necessary to additionally apply as teaching the same general steps of recovery].

1. Eggink et al. I (entire document) teach:

"Method for producing a biologically degradable polyhydroxyalkanoate coating with the aid of an aqueous dispersion of polyhydroxyalkanoate" (title):

"A method for producing a biologically degradable polyhydroxyalkanoate coating in the form of an elastomeric film, wherein an aqueous dispersion of polyhydroxyalkanoate or a mixture of polyhydroxyalkanoate is prepared and the dispersion is applied to the surface to be coated, after which water is made or allowed to evaporate to obtain a polyhydroxyalkanoate film, the film formation taking place at a temperature lower than the melting point of the polyhydroxyalkanoate, wherein the polyhydroxyalkanoate is a Pseudomonas polyhydroxyalkanoate other than a polymer or copolymer of .beta.-hydroxyvalerate or .beta.-hydroxybutyrate, without requiring additional steps to render the film elastomeric" (abstract);

wherein (col. 5), Eggink et al. I teach the extraction of PHA's, but that PHB does not necessarily have to be extracted as part of the method, but is otherwise known by routine methods to be extractable:

PHB and the copolymer PHB/HV, the polyhydroxyalkanoates of the PHB type, are typically thermoplastics having a high melting point (120-180.degree. C.) (Byrom 1987, TIBTECH 5, 246-250). These polymers are highly crystalline. PHAs formed by Pseudomonades, in particular by fluorescent Pseudomonades belonging to the RNA homology group I have, on the other hand, typically elastomeric properties. The melting point of this category of polyhydroxyalkanoates varies between 40 and 60.degree. C. and the crystallinity depends on the composition, but is between 0 and 30% (Marchessault et al. 1990, Ind. J. Biol. Macromol. 12, 158-165). Pseudomonas strains can easily be brought in a known manner to high production levels of PHA (Eggink et al. 1992, Proceedings of ACS Division of Polymeric material 67, 130-131). Pseudomonas produces no PHB, and therefore, the

polyhydroxyalkanoates which are formed by Pseudomonas can be used in an outstanding way in a method according to the present invention without first extracting PHB therefrom. In particular, because the working up of polyhydroxyalkanoates from microorganisms provides an aqueous dispersion, mixtures of polyhydroxyalkanoates occurring in microorganisms, in particular, are extremely suitable for use in a method according to the present invention. The working-up and coating can be performed continuously and in a minimum number of steps. As already stated, the PRA-containing bacteria cells can be broken open by means of a combined physical, chemical and enzymatic procedure, as a result of which the PHA granules are released from the biomass and an aqueous dispersion is produced which can be purified further from contaminating constituents by means of washing steps and centrifugation. With the present method, spray drying of the dispersion and adding of organic solvents are no longer necessary. The processing of PHA to form a coating can be (carried out) just by applying the dispersion as it is obtainable directly by working-up from the microorganisms and by allowing it to dry. The polyhydroxyalkanoates of the PHB type probably do not form an impermeable film because of the high crystallinity and the high glass transition temperature of 0-4.degree. C. the invention in all embodiments (method of preparation, film, coated product, latices) is preferably directed at polyhydroxyalkanoates having a glass transition temperature below 0.degree. C.

2. Eggink et al. II (US 5958480) (entire document) teach:

"Method for producing a biologically degradable polyhydroxyalkanoate coating with the aid of an aqueous dispersion of polyhydroxyalkanoate" (title);

"A method for producing a biologically degradable polyhydroxyalkanoate coating in the form of an elastomeric film, wherein an aqueous dispersion of polyhydroxyalkanoate or a mixture of polyhydroxyalkanoate is prepared and the dispersion is applied to the surface to be coated, after which water is made or allowed to evaporate to obtain a polyhydroxyalkanoate film, the film formation taking place at a temperature lower than the melting point of the polyhydroxyalkanoate, wherein the polyhydroxyalkanoate is a Pseudomonas polyhydroxyalkanoate other than a polymer or copolymer of .beta.-hydroxyvalerate or .beta.-hydroxybutyrate, without requiring additional steps to render the film elastomeric" (abstract);

wherein (col. 5), Eggink et al. I teach the extraction of PHA's, but that PHB does not necessarily have to be extracted as part of the method, but is otherwise known by routine methods to be extractable:

PHB and the copolymer PHB/HV, the polyhydroxyalkanoates of the PHB type, are typically thermoplastics having a high melting point (120-180.degree. C.) (Byrom 1987, TIBTECH 5, 246-250). These polymers are highly crystalline. PHAs formed by Pseudomonades, in particular by fluorescent Pseudomonades belonging

to the RNA homology group I have, on the other hand, typically elastomeric properties. The melting point of this category of polyhydroxyalkanoates varies between 40 and 60.degree. C. and the crystallinity depends on the composition, but is between 0 and 30% (Marchessault et al. 1990, Ind. J. Biol. Macromol. 12, 158-165). Pseudomonas strains can easily be brought in a known manner to high production levels of PHA (Eggink et al. 1992. Proceedings of ACS Division of Polymeric Material 67, 130-131). Pseudomonas produces no PHB, and therefore, the polyhydroxyalkanoates which are formed by Pseudomonas can be used in an outstanding way in a method according to the present invention without first extracting PHB therefrom. In particular, because the working up of polyhydroxyalkanoates from microorganisms provides an aqueous dispersion, mixtures of polyhydroxyalkanoates occurring in microorganisms, in particular, are extremely suitable for use in a method according to the present invention. The working-up and coating can be performed continuously and in a minimum number of steps. As already stated, the PHA-containing bacteria cells can be broken open by means of a combined physical, chemical and enzymatic procedure, as a result of which the PHA granules are released from the biomass and an aqueous dispersion is produced which can be purified further from contaminating constituents by means of washing steps and centrifugation. With the present method, spray drying of the dispersion and adding of organic solvents are no longer necessary. The processing of PHA to form a coating can be (carried out) just by applying the dispersion as it is obtainable directly by working-up from the microorganisms and by allowing it to dry. The polyhydroxyalkanoates of the PHB type probably do not form an impermeable film because of the high crystallinity and the high glass transition temperature of 0-4.degree. C. the invention in all embodiments (method of preparation, film, coated product, latices) is preferably directed at polyhydroxyalkanoates having a glass transition temperature below 0.degree. C.

3. **Noda et al. (US 5942597)** teach (entire document):

"Solvent extraction of polyhydroxyalkanoates from biomass" (title)

Co. 1:

"Solvent extraction of polyhydroxyalkanoates from biomass" (title); "The present invention relates to methods of extracting specific components from other biomass components. More specifically, the present invention relates to the extraction of a polyhydroxyalkanoate from a biological system, such as a plant or bacteria, by performing the extraction with a solvent".

4. **Noda (US 5821299)** teach (entire document):

"Solvent extraction of polyhydroxy-alkanoates from biomass facilitated by the use of marginal nonsolvent" (title)

Col.'s 1-2:

"The present invention relates to methods of extracting specific components from other biomass components. More specifically, the present invention relates to the extraction of a polyhydroxyalkanoate from a biological system, such as a plant or bacteria, by performing the extraction with a solvent; the extraction process being facilitated by the use of a marginal nonsolvent for PHA."

[]

"Known solvent extraction methods are also limited for a large-scale separation of PHA from a biomass. A commonly used solvent for the extraction of PHA from bacteria is chloroform. Also described for use are other halogenated hydrocarbon solvents such as dichloromethane, dichlorethane and chloropropane (see, e.g., U.S. Pat. No. 4,562,245, Stageman, issued Dec. 31, 1985; U.S. Pat. No. 4,324,907, Senior, Wright and Alderson, issued Apr. 13, 1982; U.S. Pat. No. 4,310,684, Vanlautem and Gilain, issued Jan. 12, 1982; U.S. Pat. No. 4,705,604, Vanlautem and Gilain, issued Nov. 10, 1987; European Patent Application 036 699, Holmes and Wright, published Sep. 3, 1981; and German Patent Application 239 609, Schmidt, Schmiechen, Rehm and Trennert, published Jan. 10, 1986). In the process of stripping the solvent, the concentrated PHA solution often forms a very high viscosity fluid or sometimes even a gel; which can be extremely difficult to process. Furthermore, such solvents are potentially harmful to health and environment if not fully removed from the PHA. Consequently, the use of a large amount of such solvents resulting in the formation of highly viscous solutions or gels, especially near the harvesting site, would be undesirable."

Drawing Description Text:

"The present invention is exemplified in schematic form in FIG. 1. This process enables one to obtain the advantages of a marginal nonsolvent for PHA (e.g., oil) for the precipitating PHA, even when the marginal nonsolvent for PHA is not present in the starting biomass (e.g., non-oilseed biomass, or bacteria). The marginal nonsolvent for PHA acts as a process aid by impeding the build up of excessive viscosity or gelation during the stripping of PHA solvent from the biomass."

5. Escalona et al. (US 5536419) teach (entire document):

"Procedure for extraction of polyhydroxyalkanoates from halophilic bacteria which contain them" (title)

Col. 1:

"PHAs are accumulated intracellularly by many bacteria in the form of granules. The use of these polymers as thermoplastics requires their separation from the rest of the cellular materials with an adequate level of purity. For this, numerous methods have been described based on the use of solvents and selective precipitants, which methods extract the polymer PHA from the complex mixture which constitutes the cellular biomass by means of a process of dissolution and precipitation." (col. 1).

Conclusion

Applicant's amendment (claim 44, now addressed under 103, as with the other claims) necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MAURY AUDET whose telephone number is (571)272-0960. The examiner can normally be reached on M-Th. 7AM-5:30PM (10 Hrs.).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang can be reached 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

MA, 6/20/10

/Maury Audet/
Examiner, Art Unit 1654